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R.F. Addison, P.-D. Hansen, and E.C. Wright

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HEPATIC MONO-OXYGENASE ACTIVITIES IN AMERICAN PLAICE
(HIPPOGLOSSOIDES PLATESSOIDES) FROM THE MIRAMICHI ESTUARY,
N.B.

by

R.F. Addison, P.-D. Hansen¹ and E.C. Wright²

Department of Fisheries and Oceans, Physical and Chemical
Sciences, Scotia-Fundy Region, Bedford Institute of
Oceanography, Dartmouth N.S. CANADA B2Y 4A2.

¹ Institute of Water, Soil and Air Hygiene of the Federal
Health Office, Versuchsfelde Marienfelde, Corrensplatz 1,
1000 Berlin 33, GERMANY.

Present address: Technische Universitaat Berlin, FB
14, Institute for Ecology, Department of Aquatic
Ecotoxicology, Goresallee 253, D-1000 BERLIN 37, GERMANY.

² Department of Pharmacology, Sir Charles Tupper Medical
Building, Dalhousie University, Halifax N.S. CANADA B3H 4H7

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ABSTRACT

Addison, R.F., Hansen, P.-D. and Wright, E.C. 1991. Hepatic mono-oxygenase activities in American plaice (Hippoglossoides platessoides) from the Miramichi estuary, N.B. Can. Tech. Rep. Fish. Aquat. Sci. 1800: 18 pp.

American plaice (Hippoglossoides platessoides) were trawled at four sites in the Miramichi between Newcastle and Burnt Church in August 1990. Liver samples were analysed immediately for indices of mono-oxygenase induction which would imply the presence of certain organic contaminants, including polynuclear aromatic hydrocarbons, co-planar chlorinated biphenyls and chlorinated dibenzodioxins and dibenzofurans. Activities of ethoxyresorufin O-de-ethylase (EROD) and cyanoethoxycoumarin O-de-ethylase (CN-ECOD) were low compared to those in plaice and in other species of demersal flatfish from elsewhere, and showed no clear variation over the sampling transect. These results are consistent with the observation that during the summer there is no steep gradient in the concentrations of these organic contaminants in river sediments over the transect, and that overall, levels of such contaminants are relatively low.

RÉSUMÉ

Addison, R.F., Hansen, P.-D. and Wright, E.C. 1991. Hepatic mono-oxygenase activities in American plaice (Hippoglossoides platessoides) from the Miramichi estuary, N.B. Can. Tech. Rep. Fish. Aquat. Sci. 1800: 18 pp.

On a effectué une pêche de la plie canadienne (Hippoglossoides platessoides) au chalut à quatre endroits de la rivière Miramichi, entre Newcastle et Burnt Church. Des échantillons de foie ont été immédiatement analysés afin de relever l'induction de la monooxygénase qui indiquerait la présence de certains contaminants organiques, y compris des hydrocarbures aromatiques polycycliques, des biphényles chlorés coplanaires, des dibenzodioxines chlorées et des dibenzofuranes chlorés. L'activité de l'éthoxyresorufine O-déséthylase (EROD) et de la cyanoéthoxycoumarine O-déséthylase (CN-ECOD) était faible par rapport à celle relevée chez les plies canadiennes et d'autres espèces de poissons plats démersaux capturées ailleurs, et n'a pas montré de variation nette dans tout le transect d'échantillonnage. Les résultats obtenus sont compatibles avec l'observation que, pendant l'été, il ne'existe pas de gradient marqué des concentrations de ces contaminants organiques dans les sédiments fluviaux du transect et, qu'en général, leurs teneurs sont relativement faibles.

INTRODUCTION

The lower part of the Miramichi River and its estuary are moderately populated and industrialised. There are two large towns (Newcastle and Chatham) and several smaller communities with a total population approaching 50,000. There are also several industries including pulp mills, an oil burning electric power plant which operates occasionally, and a wood preserving plant which operated until the 1970s. Many local residents believe that the Miramichi River and estuary are polluted, at least partly as a result of this concentration of population and industrial activity, and that pollution has contributed to declines in the Miramichi salmon population.

The distribution and dynamics of sediments and sediment-associated metals in the Miramichi from Newcastle downstream was studied extensively in the late 1970s and early 1980s (Willey and Fitzgerald, 1980; Buckley and Winters, 1983). These data show a gradual decline in sediment metal concentrations downstream from Newcastle. However, the gradient in metal distribution varied with season depending on the influence of spring run-off, and on tidal movements. Fewer data exist describing organic contaminants; most are relatively recent and were acquired to support Ocean Dumping Control Act regulations which govern dredging of a shipping channel (K.Tay, EPS, Dartmouth, N.S., personal communication). These data do not record any high contaminant levels or steep gradients in organic chemical concentrations downstream from Sheldrake Is. (Fig. 1). A recent compilation of all the data obtained since the late 1970s to support the dredging operation also shows that there is no steep organic contaminant gradient,

as illustrated by polychlorinated biphenyl (PCB) concentrations in sediments, downstream from Newcastle (McKnight, 1990).

Measurements of fish hepatic mono-oxygenase activity in resident flatfish are a reasonably good indicator of the presence and effects of certain organic chemicals, including polynuclear aromatic hydrocarbons (PAH), some PCBs and some chlorinated dibenzofurans and dibenzodioxins (Addison and Payne 1986; Addison and Edwards, 1988). As these chemicals are among those expected to contaminate the Miramichi estuary, we have examined hepatic mono-oxygenases in local fish with the aim of defining the zone of impact of any contamination by these chemicals.

EXPERIMENTAL

SAMPLING SITES

When this study was planned, the only recent data available describing contaminant levels in sediments downstream from Newcastle dealt with the region downstream from Sheldrake Is. (Fig. 1, 47° 05' N, 65° 19' W, approx.). In general, contaminant levels downstream from Sheldrake Is. were fairly low: Cd, for example, was consistently less than 1 $\mu\text{g g}^{-1}$ dry wt, which is considered close to "background" levels (Swiss and Henderson, 1980). Similarly, PCBs were in the range of 40 $\mu\text{g kg}^{-1}$ which is also typical of "background" levels (Addison, 1980). There appeared to be a very slight decline in the distribution of these contaminants downstream from Sheldrake Is. to the barrier islands at the mouth of the estuary.

In the light of these data, four sampling sites were

selected:

(i) east of Burnt Church in the north of Horse Shoe Shoal, in the area 47° 10' - 12' N, 65° 05' - 06' W --- described below as "Burnt Church";

(ii) south of Shel Drake Is., 47° 05' N, 65° 19' W ("Shel Drake Is.");

(iii) off the New Brunswick Electric Power plant at Chatham and west of Middle Is. in the area 47° 02' - 3' N, 65° 27' - 28' W ("NB EP");

(iv) downstream from Morrissey Bridge, Newcastle, in the area 47° 00' - 01' N, 65° 32' - 33' W ("Newcastle").

Sites upstream of Newcastle were inaccessible as Morrissey Bridge was closed to shipping.

FISH SAMPLING

Anecdotal evidence from local fishermen and data from the DFO salmon counting station at Millbank (D. Moore, DFO, Moncton N.B., personal communication) suggested that various species of flatfish were available at some points on the river, though these fish might migrate seasonally. Fish were caught on August 8 - 9 1990 using a 2 m beam trawl towed at <2 kts by a 18 m charter vessel. Trawls were no longer than 15 min and were in water depths of 5 - 15 m.

ANALYSIS OF MONO-OXYGENASE ACTIVITY

As many analyses as possible were carried out on the charter vessel between trawls. Flatfish were identified by external appearance (Leim and Scott, 1966), weighed using an "averaging" balance (Addison and Payne 1986) and were dissected and sexed immediately after trawling. Livers were excised and weighed, and approx. 0.5 g samples were homogenised in 5 ml 1.15% KCl. Aliquots of the homogenate

were centrifuged at 10,000 x g in a Beckman micro-centrifuge run in a refrigerator. One of the aliquot supernatants was used for determination of ethoxyresorufin O-de-ethylase (EROD) and cyanoethoxycoumarin O-de-ethylase (CN-ECOD) aboard the vessel (Addison and Payne, 1986; White, 1988); excitation and emission wavelengths for CN-ECOD were 400 and 455 nm respectively. The unused portion of this aliquot was stored in liquid nitrogen for protein determinations at BIO using the Pierce BCA reagent (Anon., 1989).

RESULTS AND DISCUSSION

Five trawls were made in the Burnt Church area. Most yielded large numbers of small shrimps and juvenile lobsters (which were returned to the sea) and a small number flatfish identified as American plaice (Hippoglossoides platessoides) which were analysed. At Sheldrake Is., one trawl was made: this yielded a large number of mussels (returned) and several flatfish, including American plaice. Two trawls off NB EP yielded debris (old oil drums and tree trunks) and anoxic mud, but also a variety of fish including tom-cod and eels (which were returned) and American plaice. A single trawl at Newcastle produced tom-cod, eels, salmon, juvenile halibut and winter flounder (all of which were returned) and American plaice.

Table 1 summarises the hepatic mono-oxygenase measurements made on the American plaice samples. Briefly, all the individuals were relatively small, though except for those from Newcastle, most were mature. The Newcastle samples were significantly smaller than those from the other sites. The gross appearance of most fish was healthy, but

one sample from off NB EP had an apparent tumour on the gut, and another from Newcastle had a lesion on its ventral exterior.

There were no obvious differences either in liver weight as % body weight or in liver protein content. However, these calculations are based on weighings of samples of approx 1 g tissue made on a moving vessel, and are subject to large errors: hence they are at best approximate, and are included only to illustrate the range of values observed.

The two enzyme activities, EROD and CN-ECOD, are based on protein determinations made in the shore laboratory and calculated from accurate volumetric measurements. We can consider these data in the context of spatial variation within the Miramichi, and in comparison with data from other areas.

There was no dramatic spatial variation in EROD and CN-ECOD between any of the sites. Results from Burnt Church may have been among the higher, and from Newcastle among the lower of the data, but the differences were not consistent. However, this trend is the opposite to what would be expected from a declining downstream gradient in contamination: higher mono-oxygenase activities should have been recorded from upstream than downstream sites. Such trends have been observed in an industrialised fjord in Norway (Addison and Edwards, 1988), in Sydney Hbr. N.S. (R.F. Addison and D.E. Willis, unpublished data) and along a contaminant gradient in the Forth estuary (Sulaiman et al., 1991). Taken together, the mono-oxygenase activities observed in the Miramichi fish suggest that there is little variation in the extent of contamination over the sites examined.

The only directly comparable data from American plaice caught in other areas are from fish caught in September 1990 at George's Bay, N.S., which is free from any industrial contamination. These fish were slightly larger (221 ± 82 g, mean $\pm$ s.d., $n = 7$) than those from the Miramichi and liver protein concentrations were higher. However, EROD activities on a liver wet weight basis were similar in both groups (6.3 ± 2.7 nmoles min^{-1} g^{-1} liver in Georges Bay (mean $\pm$ s.d., $n = 7$) v. a range of $3.5 - 10.4$ nmoles min^{-1} g^{-1} liver at the four Miramichi sites). CN-ECOD activity on a fresh weight basis was higher in Georges Bay (11.7 ± 0.2 nmoles min^{-1} g^{-1} ; mean $\pm$ s.d., $n = 7$) than in the Miramichi ($0.04 - 0.4$ nmoles min^{-1} g^{-1}). It is difficult to compare EROD and CN-ECOD activities in American plaice directly with data from other species, but the activities observed in the Miramichi samples are at the low end of the ranges found in other benthic flatfish such as winter flounder (Pseudopleuronectes americanus: Edwards et al, 1988) European flounder (Platichthys flesus: Addison and Edwards, 1988) and dab (Limanda limanda: R.F. Addison, unpublished). Taken together, these data suggest that the Miramichi fish are not appreciably stressed by the organic contaminants listed above.

The question arises as to whether migratory movements of American plaice in the Miramichi might have blurred any differences in mono-oxygenase activity expected from spatial differences in contamination. Unfortunately, nothing appears to be known about the movements of this species in the Miramichi. However, in similar studies, other flatfish species sampled from sites 10 - 15 km apart had clearly different mono-oxygenase activities (Addison and Edwards, 1988; R.F. Addison and D.E. Willis, unpublished data). The

approximate distances between the sites studied in this work are:

Burnt Church to Shel Drake Is:	20 km;
Shel Drake Is. to NB EP:	14 km;
NB EP to Newcastle:	11 km.

It seems unlikely that there would be any continuous or rapid mixing of groups of fish from these sites.

The absence of any clear spatial variation in MFO activity is consistent with data describing organic contaminant distribution in the Miramichi. Detailed analyses of contaminants in sediments showed that PCB concentrations (for example) did not vary much between the sites chosen for the mono-oxygenase measurements reported here (S. McKnight, pers. comm.). Thus, PCB concentrations at Newcastle ranged from 0 (undetectable) - $12 \mu\text{g} \cdot \text{kg}^{-1}$ (1977 grab samples); at the site closest to NB EP, a single grab sample (1977) contained $33 \mu\text{g} \cdot \text{kg}^{-1}$, and at the Shel Drake Is. site, concentrations ranged from 8 - $75 \mu\text{g} \cdot \text{kg}^{-1}$ (1977 and 1988 samples). All these concentrations are similar to, or below, sediment concentrations considered to reflect "background" contamination. Although there were no data for PAH at these sites, it is expected that the distribution of PAH would roughly follow that of PCBs, since it is controlled by similar physico-chemical factors. No chemical analyses were available for sediments from the Burnt Church area. This relatively uniform distribution of contaminants in the estuary and inner bay probably results from extensive sediment transport caused by tidal movements (Buckley and Winters, 1983; Winters, 1983).

CONCLUSIONS

Hepatic EROD and CN-ECOD activities in American plaice were relatively low, and roughly similar, at four sites in the Miramichi from Newcastle downstream to near the barrier islands at the mouth of the inner bay. This suggests that there is no dramatic spatial variation in the distribution of contaminants which would cause mono-oxygenase induction; these contaminants include polycyclic aromatic hydrocarbons, co-planar PCBs or planar chlorinated dibenzodioxins and dibenzofurans. These data support chemical analyses of sediments sampled from various points in the estuary and inner bay, and imply that the river from Newcastle downstream is not highly contaminated by these compounds.

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Table 1
Indices of hepatic mono-oxygenase activity in American plaice (*Hippoglossoides platessoides*) from sites in the Miramichi River sampled in Aug 8 - 9 1990.

Location	Weight g	Length cm	Sex	Comment	Liver % body wt. (approx.)	PMS protein mg g ⁻¹ liv. (approx.)	EROD ^a pmol min ⁻¹ mg prot ⁻¹	CN-ECOD ^b pmol min ⁻¹ mg prot ⁻¹
Burnt Church n=5	Mean	21.24	4F,		1.52	43.16	240.88 ^a	8.87 ^a
	S.D.	31.92	1 imm		0.81	57.42	103.77	6.29
Sheldrake Is. n=7	Mean	136.19 ^a	6F,		1.29	27.17	128.97 ^a	3.02 ^a
	S.D.	55.79	1M		0.71	14.75	73.12	1.88
NB EP n=6	Mean	129.67 ^a	5F,	1 gut	1.04	22.01	247.83 ^a	4.33 ^a
	S.D.	35.76	1M	tumour?	0.18	3.97	110.14	1.84
Newcastle n=8	Mean	85.38 ^a	2F, 2M	1 ventral	1.06	20.38	180.13 ^a	2.20 ^{bc}
	S.D.	7.91	4 imm	tumour?	0.30	6.62	127.18	2.05

Data in the same column with different superscripts are significantly different ($P < 0.05$)

^aEROD, ethoxoresorufin O-de-ethylase; CN-ECOD, cyanohydroxycoumarin O-de-ethylase.

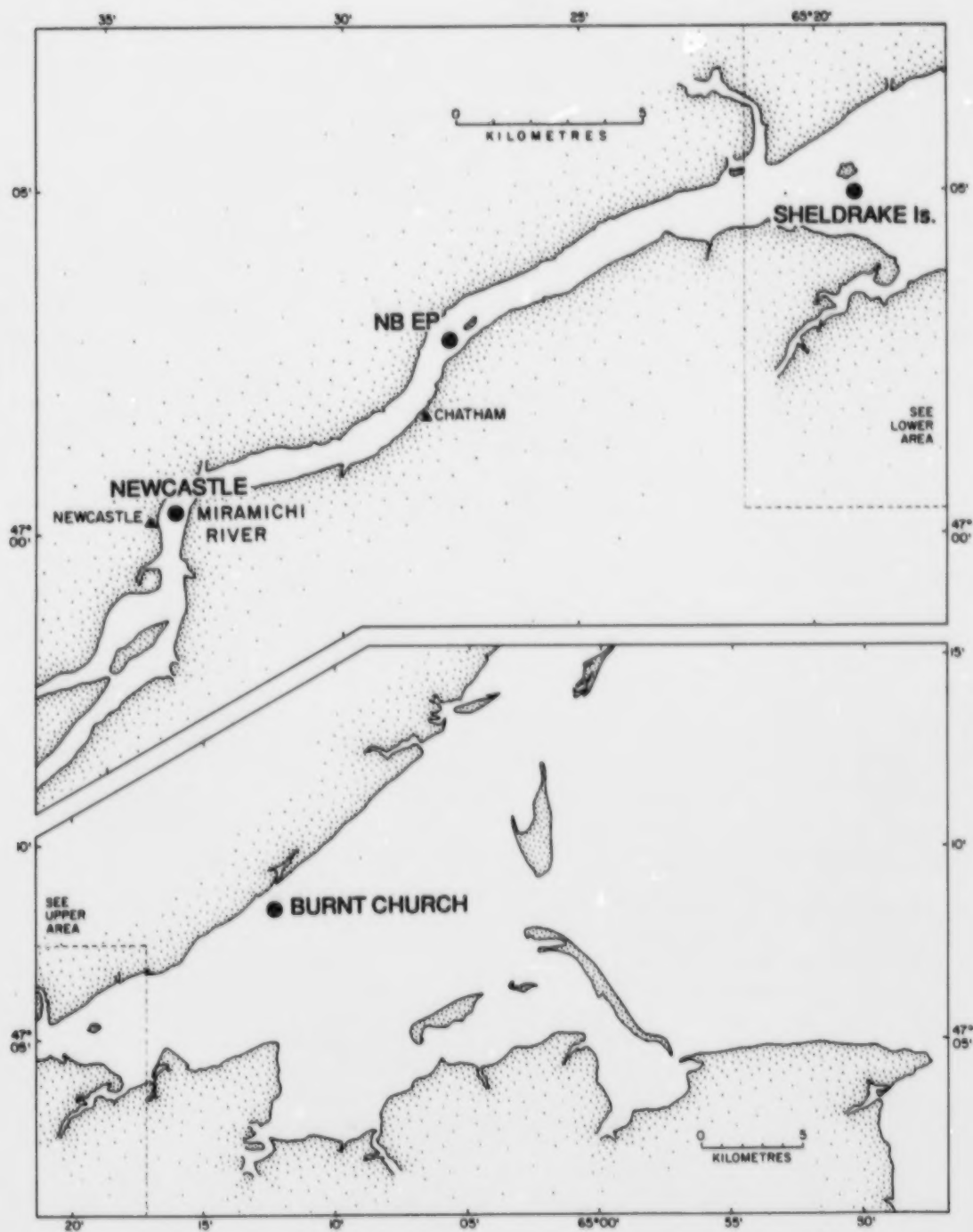


Fig. 1. Map of the lower Miramichi River and estuary showing the approximate position of the sampling sites described in text.